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User Manual

OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP (ESCs/GFP)

Cat. No. MUAES-01101

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CONTENTS AND STORAGE

Product Name	Strain 129 Mouse Embryonic Stem Cells With GFP
Catalog No.	MUAES-01101
Amount per Vial	1×10^6 Cells
Cryopreserved At	The 23rd Passage
Storage Condition	Liquid Nitrogen

CAUTION: **Please handle this product as a potentially biohazardous material. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material, in the freezing medium.**

PRODUCT INTRODUCTION

Embryonic Stem Cells With GFP (ESCs/GFP) are pluripotent cells derived from the inner cell mass of blastocysts. These cells are able to differentiate into all derivatives of the primary germ layers, including ectoderm, endoderm, and mesoderm, thus generating every cell type in the body. Different from most other stem cells, ESCs/GFP are capable of self-renewal indefinitely. Because of their plasticity and potentially unlimited capacity for self-renewal, ES cell therapies have been proposed for regenerative medicine and tissue replacement.

OriCell™ Strain 129 Mouse ESCs/GFP maintain diploid karyotype after extended passages *in vitro.* These cells express specific clusters of different proteins for ESCs/GFP, and are capable of forming embryoid bodies *in vitro* and developing teratomas in nude mice.

Cyagen OriCell™ Strain 129 Mouse ESCs/GFP are derived from the inner cell mass of strain 129 mouse blastocysts (at 3.5 dpc) and cultured on γ-ray irradiated mouse embryonic fibroblasts as feeder cells in OriCell™ Mouse ESC Growth Medium, and then have been transfected with a lentiviral construct containing a GFP expression motif, and been selected from a purified ESCs/GFP clone.

In addition, these cells have been tested for:

- Exogenous Factors: bacterial/fungal contamination, mycoplasma contamination, and endotoxin contamination.
- Characteristics: post-thaw viability, cell cycle, verification of undifferentiated state, and differentiation potential.

This product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

CELL CHARACTERISTICS AND IDENTITY

- Ability to differentiate into all derivatives of the three primary germ layers.
- Reproduce indefinitely under proper conditions.
- Positive for pluripotent stem cell markers Oct4, SSEA-1 and Nanog (⩾ 90%), negative for SSEA-3 and SSEA-4 (≤ 5%)

PRODUCT APPLICATIONS

OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP (ESCs/GFP) are potent tools for basic and applied research in diverse fields, including basic mechanism involved in developmental procedure and disorder, regenerative biology and potential therapies. Specially, ESCs/GFP are a valuable utility to make genetically modified mice by introducing mutations into the mouse germ line.

GENERAL HANDLING PRINCIPLES

1. Aseptic handling of the product is necessary throughout.
2. Once the cells have been established, always freeze several vials of OriCell™ 129 Mouse ESCs/GFP as a backup.
3. Establish and maintain Cyagen OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP on mouse embryonic fibroblasts (MEFs) feeder layers. We recommend using Cyagen OriCell™ Strain ICR MEFs (Irradiated) for culturing mouse ESCs/GFP.
4. For general maintenance of cells, we recommend the seeding density to be 2.0-2.5× 10^4cells/cm^2.
5. Do not let OriCell™ 129 Mouse ESCs/GFP overgrow as it will result in contact between the colonies. We recommend that the Mouse ESCs/GFP are routinely passaged every 48 hrs.

Gelatin Coating of Tissue Culture Vessels for Mouse Embryonic Fibroblasts (MEFs)

Materials Required:

- Gelatin Solution (Cat. No. GLT-11301)

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient Gelatin Solution into the culture vessel to completely cover its base.

2. Swirl until Gelatin Solution coats the entire base of vessel. Let it sit for at least 30 minutes at room temperature.
3. Aspirate off all of the Gelatin Solution and allow the residual amount to evaporate by leaving the vessel sitting open in the laminar flow hood/biological safety cabinet for no more than 30 minutes.
4. Enclose the culture vessel once it has dried.

Note: **Gelatinized dishes or flasks can be stored at 4°C for no more than 2 weeks, provided they remain sterile.**

THAWING AND ESTABLISHING MOUSE EMBRYONIC FIBROBLASTS (MEFs)

Materials Required

- Gelatin Solution (Cat. No. GLT-11301)
- OriCell™ Strain ICR Mouse Embryonic Fibroblasts (Cat. No. MUIEF-01002)
- OriCell™ Mouse Embryonic Fibroblast Growth Medium (Cat. No. MUXEF-90011)

Thawing and Establishing MEFs

1. Pre-warm the OriCell™ MEF Growth Medium to 37°C.
2. Add 9 mL of OriCell™ MEF Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of OriCell™ Strain ICR MEFs from liquid nitrogen.
4. Quickly thaw the vial in a 37°C water bath until the last ice crystal disappears. For optimal results, be sure to finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: **Results will be less than optimal if the cells are thawed for more than 3 minutes**

5. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% v/v ethanol.
6. Use a pipette to transfer the cells to the 15 mL conical tube containing OriCell™ MEF Growth Medium inside a biosafety cabinet. Be careful not to introduce any bubbles during the transfer process.
7. Rinse the vial with 1 mL of medium to reduce cell loss. Subsequently transfer this 1mL of cell suspension to the conical tube.
8. Gently mix the cell suspension by slowly pipetting up and down. Be careful not to introduce any bubbles.
9. Centrifuge the cell suspension at 250 x *g* for 5 minutes.
10. Carefully aspirate off as much of the supernatant as possible and add 3 mL of fresh OriCell™ MEF Growth Medium (pre-warmed to 37°C).

11. Gently resuspend the cells in OriCell™ MEF Growth Medium.
12. Seed the cells into 6-well plates pre-coated with Gelatin Solution (or other appropriate flasks) and add sufficient OriCell™ MEF Growth Medium. Gently rock the culture plate to evenly distribute the cells.

Note: **We recommend the seeding density of MEFs to be 2.0-3.0×10^{4}cells/cm^2.**

13. Incubate at 37°C in a 5% CO_2 humidified incubator.
14. The next day, change the medium with fresh OriCell™ MEF Growth Medium (pre-warmed to 37°C).

Note:

1. **If the next day thawing of the Embryonic Stem Cells With GFP is performed, the medium can be changed directly to embryonic stem cell growth medium.**
2. **Thawing the feeder cells should be performed at least one day before thawing Embryonic Stem Cells With GFP.**
3. **The feeder cells should be used as soon as possible once thawed.**

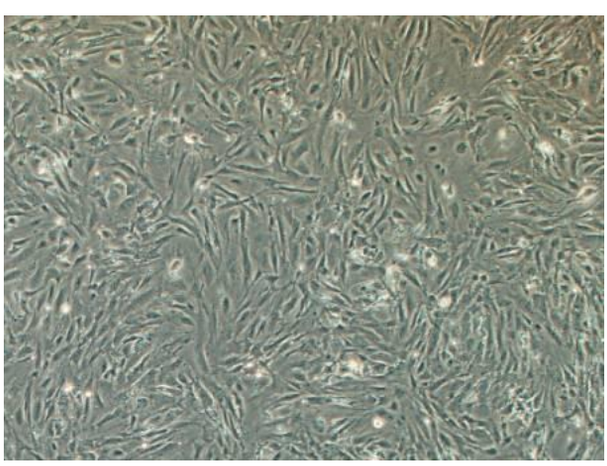

Fig. 3 Cyagen OriCell™ Mouse Embryonic Fibroblasts (Irradiated) plated on culture vessels coated with 0.1% gelatin

THAWING AND ESTABLISHING OriCell™ STRAIN 129 MOUSE EMBRYONIC STEM CELLS WITH GFP (ESCs/GFP)

Materials Required:

- Gelatin Solution (Cat. No. GLT-11301)
- OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP (Cat. No. MUAES-01101)
- OriCell™ Mouse Embryonic Stem Cell Growth Medium (Cat. No. MUXES-90011)

Thawing and Establishing 129 Mouse ESCs/GFP

1. Pre-warm the OriCell™ Mouse ESC Growth Medium and 1×PBS to 37°C.
2. Add 9 mL of OriCell™ Mouse ESC Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of OriCell™ Strain 129 Mouse ESCs/GFP from liquid nitrogen.
4. Quickly thaw the vial in 37°C water bath until the last ice piece disappears. For optimal results, be sure to finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: **Results will be less than optimal if the cells are thawed for more than 3 minutes**

5. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% v/v ethanol.
6. In a laminar flow hood, use pipette to transfer the cells to the conical tube containing OriCell™ Mouse ESC Growth Medium.

Note: **Be careful not to introduce any bubbles during the transfer process.**

7. Rinse the vial with 1 ml of medium to reduce the loss of cell and then transfer the cell suspension to the conical tube.
8. Gently mix the cell suspension by slowly pipetting up and down. Be careful not to introduce any bubbles.
9. Centrifuge the cell suspensions at 250 x *g* for 5 minutes.
10. In the meantime, aspirate the spent medium from the OriCell™ ICR Mouse Embryonic Fibroblasts (MEFs), and add the pre-warmed OriCell™ Mouse ESC Growth Medium. Return the MEFs to the 5% CO_2 humidified incubator.
11. Carefully aspirate as much of the supernatant as possible and add 3 mL of fresh OriCell™ Mouse ESC Growth Medium (pre-warmed to 37°C).
12. Gently re-suspend the cells in OriCell™ Mouse ESC Growth Medium.
13. Plate the cells into TWO T25 flasks with MEFs and add sufficient OriCell™ Mouse ESC Growth Medium. Gently rock the culture flask to evenly distribute the cells.
14. Incubate at 37°C in a 5% CO_2 humidified incubator.
15. The next day, change the medium with fresh OriCell™ Mouse ESC Growth Medium (pre-warmed to 37°C).

Note: **Changing Medium**

1. Warm an appropriate amount of OriCell™ Mouse ESC Growth Medium to 37°C in a sterile container. Remove the spent medium and replace it with the warmed, fresh medium and return the flask to the incubator.
2. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.

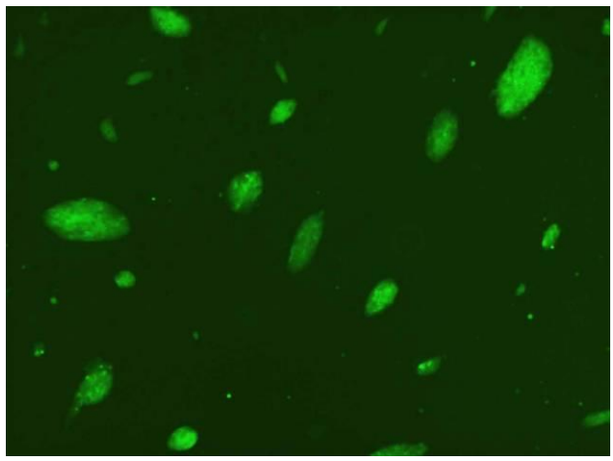

Fig.2 Image of OriCell™ Stain 129 Mouse Embryonic Stem Cells With GFP at P25 cultured on OriCell™ ICR Mouse Embryonic Fibroblasts (Irradiated) feeder cells.

PASSAGING OriCell™ STRAIN 129 MOUSE EMBRYONIC STEM CELLS WITH GFP (ESCs/GFP)

Materials Required:

- OriCell™ Mouse Embryonic Stem Cell Growth Medium (Cat. No.MUXES-90011)
- Vessels plated with MEFs

Passaging 129 Mouse ESCs/GFP

1. Pre-warm OriCell™ Mouse ESC Growth Medium, 1×PBS, Trypsin-EDTA solution to 37°C.
2. Aspirate the spent medium from the OriCell™ ICR Mouse Embryonic Fibroblasts (MEF).
3. Rinse MEF with 1×PBS (3 mL for one well of six-well plate).
4. Aspirate the 1×PBS from the flask and discard.
5. Repeat step 3-4 once or twice.
6. Add the pre-warmed OriCell™ Mouse ESC Growth Medium. Return the MEF to the 5% CO_2 humidified incubator.

Note: **Be careful not to disturb the monolayer of MEF during step 2-6.**

7. Carefully aspirate off spent medium from OriCell™ Strain 129 Mouse ESCs/GFP.
8. Rinse the cells with 1×PBS (3 mL for one well of six-well plate).
9. Aspirate the 1×PBS from the flask and discard.
10. Repeat the step 8-9 two or three times.
11. Add Trypsin-EDTA solution (200 µL for one well of six-well plate) and incubate for 1-2 minutes, until the OriCell™ Strain 129 Mouse ESCs/GFP are dissociated. At this

point, gently tap the side of the flask to release the majority of cells from the culture surface.

12. Add OriCell™ Mouse ESC Growth Medium (3 mL for one well of six-well plate) and gently pipette up and down until colonies become dissociated to single cells.

Note: **Be careful not to introduce any bubbles.**

13. Transfer the dissociated cells into a 15 mL conical tube.
14. Centrifuge the tube at 250 x *g* for 5 minutes to pellet the cells.
15. Carefully aspirate off as much of the supernatant as possible.
16. Add 2 mL of OriCell™ Mouse ESC Growth Medium to the conical tube and re-suspend the cells thoroughly but gently.
17. Plate the cells into flasks containing the MEF. Split ratios for OriCell™ Strain 129 Mouse ESCs/GFP can vary from 1:6 to 1:10. Do not exceed 1:10.
18. Add sufficient medium.
19. Incubate the cells at 37°C in a 5% CO_2 humidified incubator until it is time to split again. We typically split OriCellTM Strain 129 Mouse ESCs/GFP every other day.

Note:

1. OriCell™ Strain 129 Mouse ESCs/GFP should be plated at a density that provides an even distribution of colonies over the surface, but does not result in contact between the colonies. Differentiation can occur if the colonies are plated too densely or too sparsely.
2. OriCell™ Strain 129 Mouse ESCs/GFP should not be over-subcultured, minimize the number of passages and the length of time the cells are kept in culture. This will ensure enhanced and reproducible experimental results.

Hints

Time to Split Strain OriCell™ 129 Mouse Embryonic Stem Cells With GFP

Passage the cells before the colonies become too large and dense. When plated at the optimum density, OriCell™ Strain 129 Mouse ESCs/GFP should be passaged every 48 hours.

DIFFERENTIATION OF OriCell™ STRAIN 129 MOUSE EMBRYONIC STEM CELLS WITH GFP (ESCs/GFP)

OriCell™ Strain 129 Mouse ESCs/GFP are capable of forming embryoid bodies in vitro and teratomas in nude mice.

Materials Required:

- OriCellTM Embryoid Body (EB) Formation Medium (Cat. No. MUXES-90051)

The formation of embryoid body (EB) is the principal step in the differentiation of ES cells. When maintained in the EB formation medium and in the absence of MEF feeder layers, ES cells differentiate spontaneously, and then form three-dimensional aggregates. This structure facilitates multicellular interactions, in which cell-cell contact exists and gap juncitons may be established.

Protocol:

1. Dissociate OriCell™ Strain 129 Mouse ESCs/GFP by incubating the cells with trypsin solution at 37°C for 1-2 min.
2. Add an appropriate volume of Cyagen OriCell™ EB Formation Medium (e.g. 3 mL for each well of six-well plate) to stop reaction and gently pipette up and down until cells in colonies become single cells.
3. Transfer cell suspension into a 15 ml conical tube and centrifuge at 250 X *g* for 5 minutes to pellet the cells.
4. Carefully aspirate as much of the supernatant as possible.
5. Add appropriate amount of Cyagen OriCell™ EB Formation Medium to the conical tube and gently resuspend the cells. Plate cell suspension in 100 mm adherent dishes.
6. Incubate the adherent dishes in a 37°C incubator for 30-40 minutes to separate Mouse Embryonic Fibroblasts from OriCell™ Strain 129 Mouse ESCs/GFP.
7. Carefully collect the suspending OriCell™ Strain 129 Mouse ESCs/GFP and adjust the cell concentration to 5 x 10^5 cells/mL with OriCell™ EB Formation Medium.
8. Plate 10mL cell suspension in one 100 mm non-adherent petri dish.
9. Incubate the cells at 37°C in a 5% CO_2 humidified incubator for 5 days to form EB, and change the medium every other day.
10. Plate EB into adherent surface of gelatin coated tissue culture vessels in Cyagen OriCell™ EB Formation Medium.
11. Incubate the EB at 37°C in a 5% CO_2 humidified incubator for about 14 days. Change media every other day.
12. Stain the differentiated cells with antibodies against endodermal, mesodermal and ectodermal markers at day 14 after EB differentiation.

CRYOPRESERVATION OF OriCell™ STRAIN 129 MOUSE EMBRYONIC STEM CELLS WITH GFP (ESCs/GFP) USING OriCell™ NCR PROTEIN-FREE CRYOPRESERVATION MEDIA

OriCell™ NCR Protein-Free Cryopreservation Medium (Cat. No. NCPF-10001) is a protein-free, ready-to-use freezing medium. Its chemically-defined and protein-free formulation has been optimized to stem cells and primary cells, thus greatly enhancing the viability and integrity of these cells by protecting them from damage during the one-step freeze-thaw procedure. Unlike other conventional freezing media, which require a slow programmed freeze, this product allows the cells to be directly frozen at -80°C.

Cryopreservation

Note: **Change the culture medium with fresh growth medium 24 hours before freezing.**

1. Collect cells that are in the logarithmic growth phase. Perform a cell count to determine the viable cell density.
2. Centrifuge the cells for 3-5 minutes at 250 x *g* and 20°C. Remove and discard the supernatant using a pipette.
3. Resuspend the cell pellet in the OriCell™ NCR Protein-Free Cryopreservation Medium at a cell density of 10^5-10^6 cells/mL.
4. Dispense aliquots of the cell suspension into cryogenic storage vials that are properly labeled.
5. Place the vials directly in a -80°C freezer. After 24 hours, transfer the frozen vials to liquid nitrogen for long-term preservation.

APPENDIX

Troubleshooting

The table below lists some potential problems and solutions for culturing OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP.

Problem	Cause	Solution
Low cell recovery rate	The storage condition does not meet the requirements	Purchase a replacement and store in liquid nitrogen for long-term preservation.
	Thawing the cells takes too long time	Thaw cells for no more than 3 minutes.
	Cells are incompletely recovered after thawing	After aspirating off medium, wash the tube with culture medium twice and transfer all of the cells to the dish.
	Cells are handled roughly	Care should be taken to avoid introducing bubbles during pipetting. Also avoid vortexing and high-speed centrifugation
	Medium is not pre-warmed	Warm medium to 37℃ before recovery.
Slow cell growth	Mycoplasma contamination.	Discard the cells in question and disinfect the laboratory environment before recovering the next batch of cells.
	Over digestion	Wash the cells with PBS 2-3 times to remove serum prior to trypsinization (serum will inhibit the function of trypsin).
		Control the digestion time.
	Plating density is too low	Increase the plating density.
	MEFs have been cultured for too long	MEFs should be used up in 5-7days after recovery
Cell aging	Inappropriate serum and medium	Use Cyagen tailor-made culture media. If other serum and media products are used, please perform validation to ensure compatibility.
	Dead cells are not removed promptly	Change the medium the next day after recovery to ensure removal of all dead cells.
	Cell Contamination	Discard the cells in question, and disinfect the experimental environment before recovering.
	Plating density is too low	Some stem cells can secrete factors to support cell growth. Therefore, a certain degree of plating density must be maintained; otherwise it will lead to cell proliferation slow down, and finally cell aging.

Cell aging	Over digestion	Wash the cells with PBS 2-3 times to remove serum prior to trypsinization (serum will inhibit the function of trypsin).
		Control the digestion time.
	The passaging time is not appropriate	The cells should be subcultured when reaching 80 to 90% confluence. Or there will be contact inhibition.
Cells show spontaneous differentiation	DMSO is not completely removed during cell recovery	Wash the cells with pre-warmed medium 2-3 times during recovery.
	Plating density is too high which may result in the fusion of clones	Subculture the ESCs/GFP when ESCs/GFP aggregates are small.
	ESCs/GFP clone is too large	Lower plating density.
	MEFs have been cultured for too long	MEFs should be used up in 5-7days after recovery.
Ineffective induction of Cell differentiation	Differentiation reagents need to be optimized	Use Cyagen tailor-made differentiation medium.
	Cell passage is too high	Use cells at a low passage number.

Related products

Product	Catalog Number
Gelatin Solution	GLT-11301
OriCell™ Mouse Embryonic Fibroblasts	MUIEF-01002
OriCell™ Mouse Embryonic Fibroblast Growth Medium	MUXEF-90011
OriCell™ Strain 129 Mouse Embryonic Stem Cells With GFP	MUAES-01101
OriCell™ Mouse Embryonic Stem Cell Growth Medium	MUXES-90011
Phosphate-Buffered Saline (1×PBS)	PBS-10001
Trypsin-EDTA	TEDTA-10001
OriCellTM Embryoid Body (EB) Formation Medium	MUXES-90051
OriCell™ NCR Protein-Free Cryopreservation Medium	NCPF-10001

References

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T M Magin, J McWhir, and D W Melton. (1992) A new mouse embryonic stem cell line with good germ line contribution and gene targeting frequency. Nucleic Acids Research 20(14):3795-3796.

J A Thomson, J Kalishman, and T G Golos.(1995) Isolation of a primate embryonic stem cell line. PNAS 92: 7844-7848.

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